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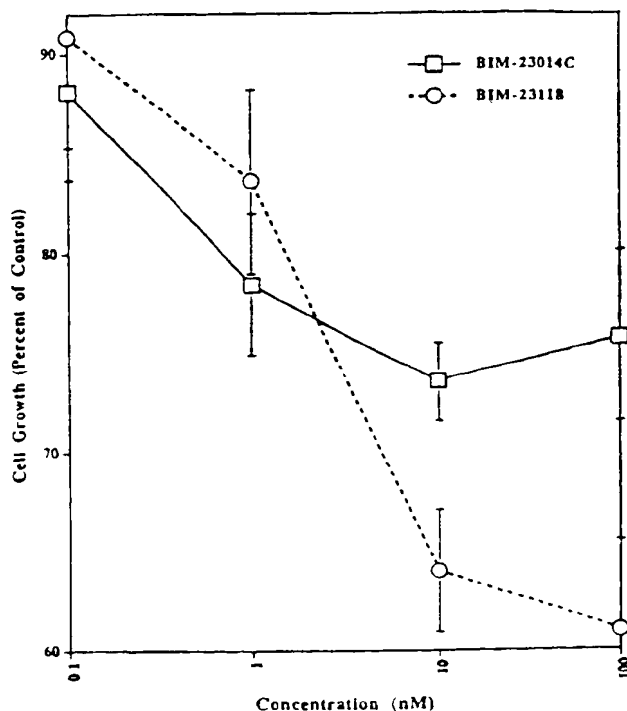
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(71) Applicant: BIOMEASURE, INC. [US/US]; 27 Maple Street, Milford, MA 01757 (US).		Published With international search report.	
(72) Inventors: KIM, Sun, Hyuk; 20 Whitney Street, Chestnut Hill, MA 02167 (US). DONG, Zhengxin; 40 Angelica Drive, Framingham, MA 01701 (US). TAYLOR, John, E.; 74 Fiske Mill Road, Upton, MA 01568 (US). MOREAU, Sylviane; 159 Westboro Road, Upton, MA 01568 (US). KEYES, Susan, Riley; Apartment 3, 41 Pinckney Street, Boston, MA 02114 (US).			
(74) Agent: CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110 (US).			

(54) Title: THERAPEUTIC PEPTIDE DERIVATIVES

(57) Abstract

Peptide derivatives containing one or more substituents separately linked by an amide, amino or sulfonamide bond to an amino group on either the N-terminal end or side chain of a biologically active peptide moiety. The peptide derivatives have relatively enhanced biological activity when compared to the corresponding peptide alone.

Effect of Somatostatin Analogs on the Proliferation of AR42J Rat Pancreatic Tumor Cells



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THERAPEUTIC PEPTIDE DERIVATIVES

Background of the Invention

This invention relates to therapeutic peptides.

5 Several attempts have been made to prolong the activity of biologically active peptides. For example, peptides have been chemically modified by synthetically adding sugar moieties to increase the period during which the peptide is active (Sandoz, WO 88/02756; Sandoz, WO
10 89/09786; DE 3910667 A1, EPO 0 374 089 A2 (1990); and Breipohl, U.S. Patent No. 4,861,755 (1989)). The addition of cationic anchors (EPO 0 363 589 A2 (1990)) and lipid moieties (Whittaker, WO 91/09837; Jung, U.S. Patent No. 4,837,303 (1989)) has also been used to
15 increase the lifetime of the peptide.

Summary of the Invention

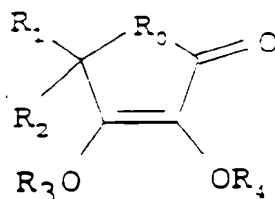
In general, the present invention provides derivatives of biologically active peptides which contain one or more substituents separately bonded to an amino
20 group located on the N-terminal end or a side chain of the peptide moiety. In this modified form, the derivatives have more potent and prolonged biological activity than the corresponding unmodified peptide.

The peptide derivatives are advantageous in that
25 they are inexpensive, highly biocompatible, lack deleterious side effects, and are compatible with different forms of therapeutic administration. In particular, many of the derivatives which have somatostatin as the peptide moiety have improved greatly
30 improved potency and selectivity compared to unmodified somatostatin.

In one aspect, the invention features a peptide derivative containing a biologically active peptide moiety and at least one substituent attached to the

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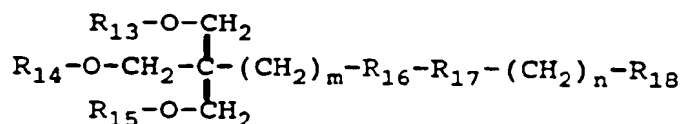
peptide moiety; the substituent is selected from the group including Compounds I, II, and III, where Compound I is:



where:

- 5 R_0 is O, S, or NR_5 , where R_5 is H or (C_1-C_6) alkyl; each R_1 and R_2 , independently, is H, $(CH_2)_mOR_6$, or $CH(OR_7)CH_2OR_8$, where R_6 is H or (C_2-C_7) acyl, and each R_7 and R_8 , independently, is H, (C_2-C_7) acyl, or $C(R_9)(R_{10})$, where each R_9 and
- 10 R_{10} , independently, is H or (C_1-C_6) alkyl; or each R_1 and R_2 is $=CHCH_2OR_{11}$, wherein in R_{11} , each R_1 and R_2 , independently, is H or (C_2-C_7) acyl, and m is an integer between 1 and 5, inclusive; and
- 15 one of R_3 or R_4 is $(CH_2)_nR_{12}$ or $(CH_2)_nCH(OH)R_{12}$, where R_{12} is CO, CH_2 , or SO_2 , and n is an integer between 1 and 5, inclusive; and the remaining R_3 or R_4 is H, (C_1-C_6) hydroxyalkyl, or (C_2-C_7) acyl; and

20 Compound II is:



where:

- 25 each R_{13} , R_{14} and R_{15} , independently, is H or (C_2-C_{24}) acyl;
- R_{16} is NH or absent;

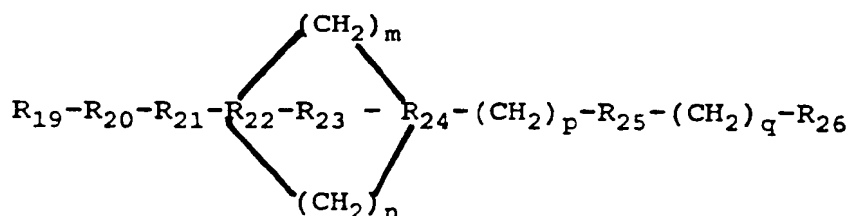
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R_{17} is CO, O, or absent;

R_{18} is CO, CH_2 , SO_2 , or absent; and

m is an integer between 1 and 5, inclusive; and n is an integer between 0 and 5, inclusive; and

5 Compound III is:



where:

10 R_{19} is H, NH_2 , an aromatic functional group, OH, (C_1-C_6) hydroxyalkyl, $\text{H}(\text{R}_{27})(\text{R}_{28})$, SO_3H , or absent where each R_{27} and R_{28} , independently, is H or (C_1-C_6) alkyl;

R_{20} is O or absent;

15 R_{21} is (C_1-C_6) alkyl or absent;

R_{22} is N, CH, O, or C;

$-R_{23}-$ is (C_1-C_6) alkyl or absent;

R_{24} is N, CH, or C;

R_{25} is NH, O, or absent;

20 R_{26} is SO_2 , CO, CH_2 , or absent;

m is an integer between 0 and 5, inclusive;

n is an integer between 0 and 5, inclusive;

p is an integer between 0 and 5, inclusive; and

q is an integer between 0 and 5, inclusive.

25 In Compounds I, II and III the peptide moiety is attached to each of the substituents by a CO-N, $\text{CH}_2\text{-N}$, or $\text{SO}_2\text{-N}$ bond between the substituent and a nitrogen atom of the N-terminus or a side chain of said peptide moiety.

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In preferred embodiments, $-R_{23}-$ is (C_1-C_6) alkyl; R_{22} is N, C or CH ; and R_{24} is C. Alternatively, R_{22} is O; R_{19} , R_{20} , R_{21} , and $-R_{23}-$ are absent; and the sum of m and n is 3, 4, or 5.

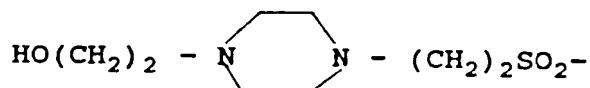
- 5 In other preferred embodiments of the invention, the substituent is Compound I; in this embodiment, R_{12} is preferably CH_2 or SO_2 . Alternatively, the substituent may be Compound II, in which case R_{18} is preferably CH_2 or SO_2 ; R_{13} , R_{14} , and R_{15} are H; and R_{17} is absent. In
10 particularly preferred embodiments, the substituent is $(HOCH_2)_3C-NH-(CH)_2-SO_2$ or $(HOCH_2)_3C-CH_2$.

In still other embodiments of the invention, the substituent is Compound III; preferably, in this
15 embodiment, $-R_{23}-$ is absent and at least one of R_{22} and R_{24} is N. Alternatively, both R_{22} and R_{24} may be N.

In other embodiments, the substituent is one of:



and



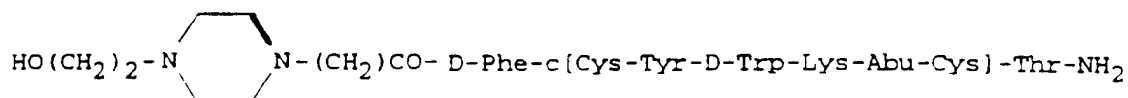
- 20 Preferably, the peptide moiety is selected from the group including: somatostatin, bombesin, calcitonin, calcitonin gene related peptide (CGRP), amylin, parathyroid hormone (PTH), gastrin releasing peptide (GRP), melanocyte stimulating hormone (MSH),
25 adrenocorticotrophic hormone (ACTH), parathyroid related peptide (PTHrP), luteinizing hormone-releasing hormone (LHRH), growth hormone releasing factor (GHRF), growth hormone releasing peptide (GHRP), cholecystokinin (CCK), glucagon, Bradykinin, glucagon-like peptide (GLP),
30 gastrin, enkephalin, neuromedins, endothelin, substance P, neuropeptide Y (NPY), peptide YY (PYY), vasoactive

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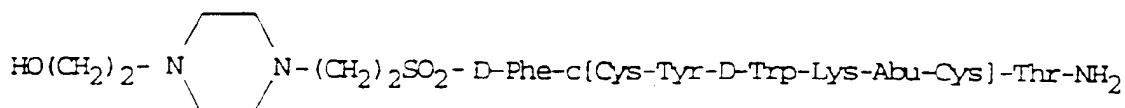
intestinal peptide (VIP), guanylin, pituitary adenylate cyclase activating polypeptide (PACAP), beta-cell tropin, adrenomedulin, and derivatives, fragments, and analogs thereof.

- 5 The peptide moiety is preferably somatostatin or a derivative, fragment, or analog thereof. Most preferably, the somatostatin analog is one of: H-D-Phe-c[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH₂, H-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH₂, and H-D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH₂. Alternatively, the peptide moiety
- 10 is bombesin or a derivative, fragment or analog thereof.

 In still other preferred embodiments, the peptide derivative is one of:



15 and



- In another aspect, the invention provides a dimeric peptide derivative containing two biologically active peptide moieties, and at least one substituent
- 20 attached to each of the peptide moieties. The substituent is selected from the group consisting of compounds IV and V, where compound IV has a generic structure equivalent to compound I, and compound V has a generic structure equivalent to compound III. In the
- 25 dimer, each of the peptide moieties is attached to the substituents by a CO-N, CH₂-N, or SO₂-N bond between the substituent and a nitrogen atom of the N-terminus or a side chain of one of the peptide moieties.

 In yet another aspect, the invention provides a

30 method for treating a disease, such as cancer, in a

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patient; the method includes the step of administering to the patient a therapeutic amount of the peptide derivatives described herein. In preferred embodiments, the peptide moiety used in the treatment is somatostatin.

5 By "biologically active", as used herein, is meant a naturally occurring, recombinant, and synthetic peptide having physiological or therapeutic activity. In general, this term covers all derivatives, fragments, and analogs of biologically active peptides which exhibit a
10 qualitatively similar or opposite effect to that of the unmodified peptide.

Brief Description of the Drawings

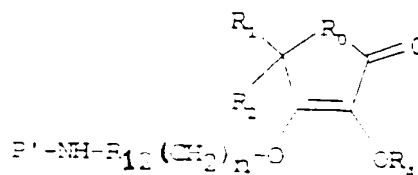
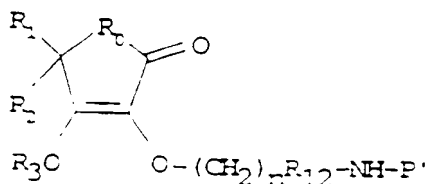
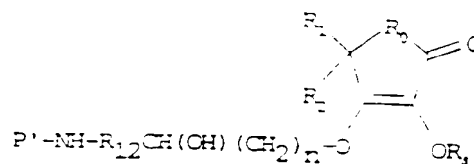
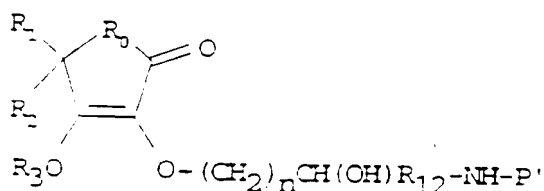
Fig. 1 is a graph of two growth curves of AR42J cells in the presence of different somatostatin
15 derivatives.

Description of the Preferred Embodiments

Peptide Derivatives

In general, peptide derivatives of the invention contain two separate components: 1) a biologically active
20 peptide; and, 2) at least one substituent having the structure of Compounds I, II, and III. Peptide derivatives made according to the methods described herein include the following compounds.

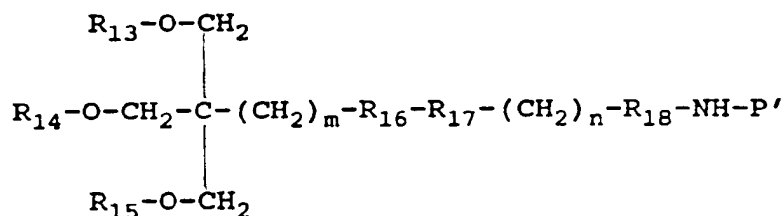
Compound I-Based Derivatives



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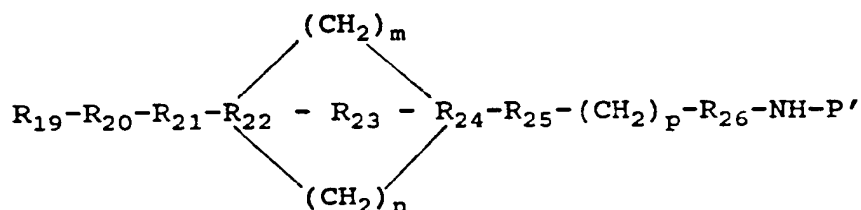
wherein R_0 , R_1 , R_2 , R_3 , R_4 , R_{12} , and n are as defined herein, and $NH-P'$ is the biologically active peptide moiety. In these embodiments, the NH group is located on the N-terminal end or side chain of the peptide and P' represents the remainder of the peptide.

Compound II-based Derivatives



10 wherein R_{13} , R_{14} , R_{15} , R_{16} , R_{17} , R_{18} , m , n , and $NH-P'$ are as defined herein.

Compound III-Based Derivatives



15

wherein R_{19} , R_{20} , R_{21} , R_{22} , R_{23} , R_{24} , R_{25} , R_{26} , m , n , p , and $NH-P'$ are as defined herein.

In addition to the structures shown above, compounds made according to the invention include peptide derivatives containing two or more substituents attached to one peptide moiety. These embodiments of the invention are derivatives of biologically active peptides which have more than one free amino group, e.g., a lysine residue.

25 The invention also provides dimeric peptide derivatives containing two peptide moieties bound to a

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single substituent, e.g., two Bradykinin analogs bound to a substituent of Compound V.

The peptide derivatives of the invention are derivatives of biologically active peptides selected from the following group: somatostatin, bombesin, calcitonin, calcitonin gene related peptide (CGRP), amylin, parathyroid hormone (PTH), gastrin releasing peptide (GRP), melanocyte stimulating hormone (MSH), adrenocorticotrophic hormone (ACTH), parathyroid related peptide (PTHrP), luteinizing hormone-releasing hormone (LHRH), growth hormone releasing factor (GRF), growth hormone releasing peptide (GHRP), cholecystokinin (CCK), glucagon, bradykinin, glucagon-like peptide (GLP), gastrin, enkephalin, neuromedins, endothelin, substance P, neuropeptide Y (NPY), peptide YY (PYY), vasoactive intestinal peptide (VIP), guanylin, pituitary adenylate cyclase activating polypeptide (PACAP), beta-cell tropin, adrenomedulin, or derivatives, fragments, or analogs of any of the foregoing.

In especially preferred embodiments, the peptide moiety is somatostatin or a derivative, fragment, or analog of somatostatin. Somatostatin analogs which can be used in accordance with the present invention include, but are not limited to the following compounds:

H-D- β -Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH₂;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys- β -Nal-NH₂;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys- β -Nal-NH₂;
H-D- β -Nal-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH₂;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH₂;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr-NH₂;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr;
H-Gly-Pen-Phe-D-Trp-Lys-Thr-Cys-Thr;
H-Phe-Pen-Tyr-D-Trp-Lys-Thr-Cys-Thr;
H-Phe-Pen-Phe-D-Trp-Lys-Thr-Pen-Thr;

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- H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol;
 H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH₂;
 H-D-Trp-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH₂;
 H-D-Trp-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH₂;
 5 H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH₂;
 H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH₂;
 H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH₂;
 Ac-D-Phe-Lys^{*}-Tyr-D-Trp-Lys-Val-Asp-Thr-NH₂
 Ac-hArg(Et)₂-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-
 10 Thr-NH₂;
 Ac-D-hArg(Et)₂-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-
 NH₂;
 Ac-D-hArg(Bu)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-
 NH₂;
 15 Ac-D-hArg(Et)₂-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH₂;
 Ac-L-hArg(Et)₂-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH₂;
 Ac-D-hArg(CH₂CF₃)₂-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-
 NH₂;
 Ac-D-hArg(CH₂CF₃)₂-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-
 20 Thr-NH₂;
 Ac-D-hArg(CH₂CF₃)₂-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-
 Phe-NH₂;
 Ac-D-hArg(CH₂CF₃)₂-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-
 Thr-NHEt;
 25 Ac-L-hArg(CH₂-CF₃)₂-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-
 Thr-NH₂;
 Ac-D-hArg(CH₂CF₃)₂-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-
 Cys-Thr-NH₂;
 Ac-D-hArg(CH₂CF₃)₂-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-
 30 Cys-Thr-NHEt;
 Ac-hArg(CH₃, hexyl)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-
 Thr-NH₂;
 H-hArg(hexyl₂)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-
 NH₂;

- 10 -

- Ac-D-hArg(Et)₂-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NHEt;
Ac-D-hArg(Et)₂-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH₂;
5 Propionyl-D-hArg(Et)₂-Gly-Cys-Phe-D-Trp-Lys(iPr)-Thr-Cys-Thr-NH₂;
Ac-D-β-Nal-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Gly-hArg(Et)₂-NH₂;
Ac-D-Lys(iPr)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH₂;
10 Ac-D-hArg(CH₂CF₃)₂-D-hArg(CH₂CF₃)₂-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH₂;
Ac-D-hArg(CH₂CF₃)₂-D-hArg(CH₂CF₃)₂-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH₂;
15 Ac-D-hArg(Et)₂-D-hArg(Et)₂-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH₂;
Ac-Cys-Lys-Asn-4-Cl-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-D-Cys-NH₂;
20 Bmp-Tyr-D-Trp-Lys-Val-Cys-Thr-NH₂;
Bmp-Tyr-D-Trp-Lys-Val-Cys-Phe-NH₂;
Bmp-Tyr-D-Trp-Lys-Val-Cys-p-Cl-Phe-NH₂;
Bmp-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH₂;
H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH₂;
25 H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH₂;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-β-Nal-NH₂;
H-pentafluoro-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH₂;
Ac-D-β-Nal-Cys-pentafluoro-Phe-D-Trp-Lys-Val-Cys-Thr-NH₂;
30 H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH₂;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH₂;
H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH₂;
H-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH₂;
35 Ac-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH₂;

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- H-D-Phe-Cys- β -Nal-D-Trp-Lys-Val-Cys-Thr-NH₂;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH₂;
cyclo (Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);
cyclo (Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);
5 cyclo (Pro-Phe-D-Trp-Lys-Thr-N-Me-Phe);
cyclo (N-Me-Ala-Tyr-D-Trp-Lys-Thr-Phe);
cyclo (Pro-Tyr-D-Trp-Lys-Thr-Phe);
cyclo (Pro-Phe-D-Trp-Lys-Thr-Phe);
cyclo (Pro-Phe-L-Trp-Lys-Thr-Phe);
10 cyclo (Pro-Phe-D-Trp(F)-Lys-Thr-Phe);
cyclo (Pro-Phe-Trp(F)-Lys-Thr-Phe);
cyclo (Pro-Phe-D-Trp-Lys-Ser-Phe);
cyclo (Pro-Phe-D-Trp-Lys-Thr-p-Cl-Phe);
cyclo (D-Ala-N-Me-D-Phe-D-Thr-D-Lys-Trp-D-Phe);
15 cyclo (D-Ala-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Phe);
cyclo (D-Ala-N-Me-D-Phe-D-Thr-Lys-D-Trp-D-Phe);
cyclo (D-Abu-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Tyr);
cyclo (N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe);
cyclo (Pro-Tyr-D-Trp-4-Amphe-Thr-Phe);
20 cyclo (Pro-Phe-D-Trp-4-Amphe-Thr-Phe);
cyclo (N-Me-Ala-Tyr-D-Trp-4-Amphe-Thr-Phe);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba-Gaba);
cyclo (Asn-Phe-D-Trp-Lys-Thr-Phe);
25 cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-NH(CH₂)₄CO);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe- β -Ala);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-D-Glu)-OH;
cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe);
cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);
30 cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);
cyclo (Asn-Phe-Phe-D-Trp(F)-Lys-Thr-Phe-Gaba);
cyclo (Asn-Phe-Phe-D-Trp(NO₂)-Lys-Thr-Phe-Gaba);
cyclo (Asn-Phe-Phe-Trp(Br)-Lys-Thr-Phe-Gaba);
35 cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe(I)-Gaba);

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- cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Tyr(But)-Gaba);
 cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-
 Pro-Cys)-OH;
 cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-
 5 Pro-Cys)-OH;
 cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-
 Tpo-Cys)-OH;
 cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-
 MeLeu-Cys)-OH;
 10 cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-Phe-Gaba);
 cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-D-Phe-Gaba);
 cyclo (Phe-Phe-D-Trp(5F)-Lys-Thr-Phe-Phe-Gaba);
 cyclo (Asn-Phe-Phe-D-Trp-Lys(Ac)-Thr-Phe-NH-
 (CH₂)₃-CO);
 15 cyclo (Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
 cyclo (Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba); and
 cyclo (Orn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba)

where Lys* indicates an amide bridge formed between Lys* and Asp.

- 20 The peptide compounds listed above are described in the following references, each of which is incorporated herein by reference:

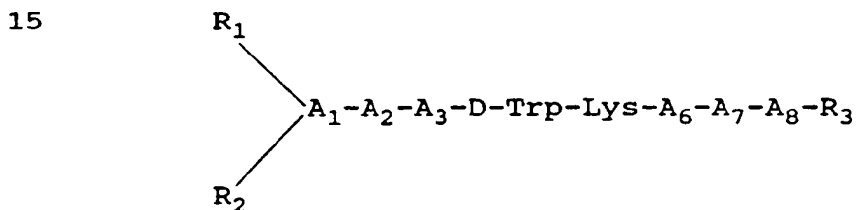
- EP Application No. P5 164 EU; Van Binst, G. et al. Peptide Research 5:8 (1992); Horvath, A. et al. Abstract,
 25 "Conformations of Somatostatin Analogs Having Anti-tumor Activity", 22nd European Peptide Symposium, September 13-19, 1992, Interlaken, Switzerland; PCT Application WO 91/09056 (1991); EP Application 0 363 589 A2 (1990); EP Application 0 203 031 A2 (1986); U.S. Patent Nos.
 30 4,904,642; 4,871,717; 4,853,371; 4,725,577; 4,684,620; 4,650,787; 4,603,120; 4,585,755; 4,522,813; 4,486,415; 4,485,101; 4,435,385; 4,395,403; 4,369,179; 4,360,516; 4,358,439; 4,328,214; 4,316,890; 4,310,518; 4,291,022;

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4,238,481; 4,235,886; 4,224,190; 4,211,693; 4,190,648;
4,146,612; and 4,133,782.

In the somatostatin analogs listed above, each amino acid residue has the structure of NH-C(R)H-CO-, in which R is the side chain; lines between amino acid residues represent peptide bonds which join the amino acids. When the amino acid residue is optically active, it is the L-form configuration that is intended unless the D-form is expressly designated. When two Cys residues are present in the peptide, a disulfide bridge is formed between the two moieties. This bond, however, is not shown in the listed residues.

Additionally preferred somatostatin analogs of the invention are of the following formula:



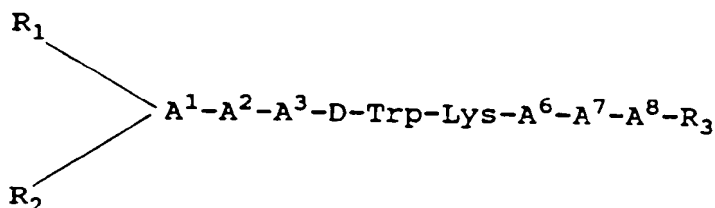
wherein A₁ is a D- or L-isomer of β-Nal, Trp, β-pyridyl-Ala, Phe, substituted Phe, or deleted; and each A₂ and A₇, independently, is Cys, Asp, or Lys. These moieties are covalently linked to each other via a disulfide bridge or an amide bridge. In addition, A₃ is β-Nal, Phe, or o-, m-, or p-substituted X-Phe where X is a halogen, OH, NH₂, NO₂ or C₁₋₃ alkyl; A₆ is Val, Thr, Ser, Ala, Phe, β-Nal, Abu, Ile, Nle, or Nva; and A₈ is Phe, Thr, Tyr, Trp, Ser, β-Nal, an alcohol group, or deleted; each R₁ and R₂, independently, is H, lower acyl or lower alkyl; and R₃ is OH, NH₂, or deleted. Preferably, when one of A₂ and A₇ is Cys, the other is also Cys; when A₈ is an alpha-amino alcohol, R₃ is deleted; and when neither of A₂ and A₇ is Cys, A₂ is different from A₇.

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Especially preferred somatostatin analogs of this embodiment are:

- 5 Me-D-Phe-Cys-Tyr-Tyr-D-Trp-Lys-Val-Cys-Thr-NH₂;
 H-D-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Nal-NH₂;
 H-D-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH₂;
 H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH₂;
 H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Nal-NH₂; and
 H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-ol.

10 In other embodiments, linear somatostatin analogs of the invention have the following structure:



wherein A¹ is a D- or L- isomer of Ala, Leu, Ile,
 15 Val, Nle, Thr, Ser, β-Nal, β-pyridyl-Ala, Trp, Phe, 2,4-dichloro-Phe, pentafluoro-Phe, p-X-Phe, or o-X-Phe, wherein X is CH₃, Cl, Br, F, OH, OCH₃ or NO₂;

A² is Ala, Leu, Ile, Val, Nle, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH₃, Cl, Br, F, OH, OCH₃ or NO₂;
 20

A³ is pyridyl-Ala, Trp, Phe, β-Nal, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH₃, Cl, Br, F, OH, OCH₃ or NO₂;

25 A⁶ is Val, Ala, Leu, Ile, Nle, Thr, Abu, or Ser;

A⁷ is Ala, Leu, Ile, Val, Nle, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH₃, Cl, Br, F, OH, OCH₃ or NO₂;

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A⁸ is a D- or L-isomer of Ala, Leu, Ile, Val, Nle, Thr, Ser, Phe, β -Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, p-X-Phe, or o-X-Phe, wherein X is CH₃, Cl, Br, F, OH, OCH₃ or NO₂, or an alcohol thereof; and

5 each R₁ and R₂, independently, is H, lower acyl or lower alkyl; and R₃ is OH, NH₂, or deleted. Preferably, at least one of A¹ and A⁸ and one of A² and A⁷ must be an aromatic amino acid; and when A⁸ is an alcohol, R₃ is deleted. Additionally, A¹, A², A⁷ and A⁸ cannot all be
10 aromatic amino acids. Particularly preferred analogs of this aspect of the invention include:

H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Thr-Phe-Thr-NH₂;
H-D-Phe-p-NO₂-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH₂;
15 H-D-Nal-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH₂;
H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH₂;
H-D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH₂;
H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH₂; and
20 H-D-Phe-Ala-Tyr-D-Trp-Lys-Val-Ala-D- β -Nal-NH₂.

In still other preferred embodiments, the peptide moiety is bombesin or a derivative, fragment, or analog of bombesin. Bombesin analogs which can be used to
25 practice the present invention include, but are not limited to, Neuromedin C, Neuromedin B, litorin, and gastrin-releasing peptide (GRP), which has the following amino acid sequence:

H-Ala-Pro-Val-Ser-Val-Gly-Gly-Gly-Thr-Val-Leu-Ala-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His- Leu-Met-NH₂
30

Other bombesin analogs which may be used in the present invention include compounds described in the

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following references, the contents of which are incorporated herein by reference:

- Coy et al. Peptides, Proceedings of the Eleventh Amer. Peptide Symposium, Ed. by Rivier et al. ESCOM, pp. 65-67 (1990); Wang et al. J. Biol. Chem. 265:15695 (1990); Mahmoud et al. Cancer Research 51:1798 (1991); Wang et al. Biochemistry 29:616 (1990); Heimbroom et al., "Synthetic Peptides: Approaches to Biological Problems", UCLA Symposium on Mol. and Cell. Biol. New Series, Vol. 86, ed. Tam and Kaiser; Martinez et al., J. Med. Chem. 28:1874 (1985); Gargosky et al., Biochem. J. 247:427 (1987); Dubreuil et al., Drug Design and Delivery, Vol 2:49, Harwood Academic Publishers, GB (1987); Heikkila et al., J. Biol. Chem. 262:16456 (1987); Caranikas et al., J. Med. Chem. 25:1313 (1982); Saeed et al., Peptides 10:597 (1989); Rosell et al., Trends in Pharmacological Sciences 3:211 (1982); Lundberg et al., Proc. Nat. Aca. Sci. 80:1120, (1983); Engberg et al., Nature 293:222 (1984); Mizrahi et al., Euro. J. Pharma. 82:101 (1982); Leander et al., Nature 294:467 (1981); Woll et al., Biochem. Biophys. Res. Comm. 155:359 (1988); Rivier et al., Biochem. 17:1766 (1978); Cuttitta et al., Cancer Surveys 4:707 (1985); Aumelas et al., Int. J. Peptide Res. 30:596 (1987); Szepeshazi. et al., Cancer Research 51:5980 (1991); Jensen, et al. Trends Pharmacol. Sci. 12:13 (1991); U.S. Patent Nos. 5,028,692; 4,943,561; 4,207,311; 5,068,222; 5,081,107; 5,084,555; EP Application Nos. 0 315 367 A2 (1989); 0 434 979 A1 (1991); 0 468 497 A2 (1992); 0 313 158 A2 (1989); 0 339 193 A1 (1989); PCT Applications Nos. WO 90/01037 (1990); 90/02545 (1992); and UK Application GB 1 231 051 A (1990).

The peptides of the invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically

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acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl
5 cellulose, and salts with inorganic acids such as hydrohalic acids, including hydrochloric acid, sulfuric acid, and phosphoric acid.

Synthesis of Compounds

The syntheses of Compounds I, II and III are now
10 described.

The following abbreviations are used in describing syntheses of compounds according to the present invention:

	Nal:	naphthylalanine (1 or 2)
15	Abu:	alpha-aminobutyric acid
	D:	dextrorotatory
	L:	levorotatory
	HOAC:	acetic acid
20	BOP:	benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluoro-phosphate
	BOC:	tert-butyloxycarbonyl
	DCC:	dicyclohexyl carbodiimide
	EDC:	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
25	DEPC:	diethylcyanophosphonate
	DMF:	dimethylformamide
	CH ₂ CL ₂ :	dichloromethane
	MeOH:	methanol
	EtOH:	ethanol
30	DIEA:	N,N-diisopropylethylamine
	HOBt:	1-hydroxybenzotriazole

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HBTU: O-Benzotriazol-1-yl,N,N,N',N'-
tetramethyluronium hexafluorophosphate

THF: Tetrahydrofuran

TFA: Trifluoroacetic Acid

5 Starting materials and intermediates for Compounds I, II, and III are commercially available. Alternatively, the starting materials can be easily prepared by methods which are well known and included in the literature. For example, the chemistry of ascorbic acid-related
10 derivatives can be found in J. Chem. Soc., Perkin Trans. 1:1220 (1974); Carbohydr. Res., 67:127 (1978); Yakugaku Zasshi, 86:376 (1966); U.S. Pat. No. 4,552,888; J. Med. Chem., 31:793 (1988); ibid. 34:2152 (1991); and, 35:1618 (1992), the contents of which are incorporated herein by
15 reference. The chemistry for tris-related derivatives can be found in Arch. Biochem. Biophys., 96, 653 (1962), Biochem., 5 467 (1966), the contents of which are also incorporated herein by reference.

Synthesis of Peptide Derivatives

20 In a general sense, the coupling of Compounds I, II, or III to an appropriate free amino group of a protected amino acid or peptide can be achieved according to well-known methods employed for peptide synthesis (e.g., DCC, DCC-HOBT, DIC-HOBT PPA, EDC-HOBT, DEPT, BOP,
25 HBTU) using a base (e.g. DIEA) in an inert solvent (e.g. DMF, THF or CH₂Cl₂ ethyl acetate or combination thereof). Deblocking of protected groups may also be carried out by well-known methods (e.g., removal of the group by the addition of acid or base, TFA, dioxan-HCl, ammonia,
30 NaOMe, piperidine). In most cases, the reaction temperature should range from -30°C to room temperature.

In general, the first step of the synthesis involves the reaction between an epoxide and a free amino

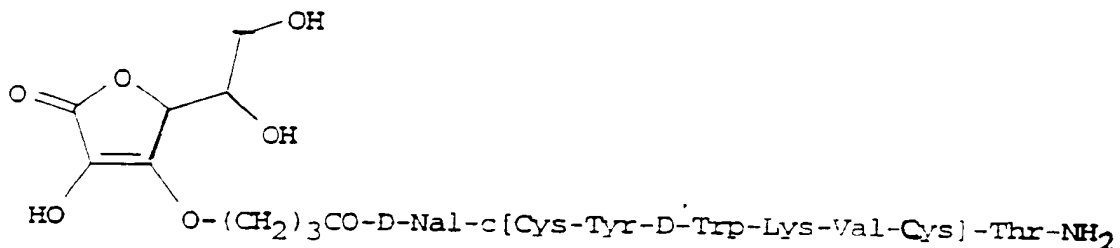
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group of a protected amino acid or peptide; complexation and deprotection can be achieved utilizing well-known methods, such as those described in McManus, et al., Synth. Communications 3, 177 (1973), the contents of which are incorporated herein by reference. Following synthesis, purification of the intermediates and products can be achieved by conventional methods such as chromatography or HPLC. The identification of the compounds may be determined by conventional techniques such as NMR, amino acid analysis, and mass spectrometry.

The following Examples illustrate the preferred methods for forming the compounds of the invention.

Example 1 - Synthesis of Somatostatin Derivatives

The following somatostatin derivative, also referred to herein as BIM-23118, was synthesized in accordance with the invention:



Example 1.1 - 3-O-(Benzyloxycarbonylmethyl)-2,5,6-triacetyl-ascorbic acid

Acetic anhydride (6 ml) was added dropwise to a solution of 3-O-(benzyloxycarbonylmethyl)-ascorbic acid (2.2 g) in pyridine (30 ml); the mixture was then stirred overnight at room temperature. Pyridine was evaporated under reduced pressure leaving a residue which was then partitioned between ethyl acetate and 1N HCl. The ethyl acetate layer was washed with 1N HCl, and then water. After drying (MgSO_4), the ethyl acetate was evaporated under reduced pressure; traces of pyridine and acetic anhydride which still remained were removed by multiple co-evaporations with toluene. The resulting 3-O-

- 20 -

(Benzyloxycarbonylmethyl)-2,5,6-triacetyl-ascorbic acid was dried under vacuum to yield a viscous gel which remained in the residue (2.4 g). TLC (silica gel: CHCl_3 /acetone [9:1], $R_f=0.52$).

5 Example 1.2 - 3-0-(carboxymethyl)-2,5,6-triacetyl-ascorbic acid

A slurry of Pd-C (100 mg) in water (2 ml) was added to a solution of 3-0-(benzyloxycarbonylmethyl)-2,5,6-triacetyl-ascorbic acid (2.4 g) in ethanol (30 ml),
10 and the suspension was shaken under hydrogen (17 psi) for six hours. The catalyst was then removed by filtration through a celite pad and the filtrate evaporated under reduced pressure to yield 3-0-(carboxymethyl)-2,5,6-triacetyl-ascorbic acid. TLC (silica gel: CHCl_3 /MeOH/HOAc
15 [9:1:0.1], $R_f=0.2$).

Example 1.3 - 5,6-0-Isopropylideneascorbic acid

Acetylchloride (0.67 ml) was added to a rapidly stirred suspension of ascorbic acid (8.0 g) in acetone (80 ml) and the mixture was stirred at room temperature
20 overnight. The precipitate was collected by filtration, washed with ethyl acetate, and dried at reduced pressure to afford 8.29 g of 5,6-0-Isopropylideneascorbic acid as a colorless solid. TLC (silica gel: CHCl_3 /MeOH/HOAc [3:1:0.1], $R_f=0.54$).

25 Example 1.4 - 3-0-(Ethoxycarbonylpropyl)-5,6-isopropylidene-ascorbic acid

A solution of 5,6-isopropylidene ascorbic acid (2.0 g) in 10 ml DMF was added dropwise to a suspension of NaH (0.44 g of 50% mineral oil NaH dispersion washed
30 with hexane several times) in 5 ml DMF. After gas evolution ceased, a solution of 1.43 ml ethyl 4-bromobutyrate in 5 ml DMF was added dropwise and the

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mixture was stirred at room temperature overnight. Solvent was evaporated at reduced pressure and the resultant residue was chromatographed on silica gel (55 g) using $\text{CHCl}_3/\text{MeOH}$ (19:1) as an eluant. Appropriate
5 fractions were pooled and solvents removed at reduced pressure to yield a viscous residue containing 3-O-(Ethoxycarbonylpropyl)-5,6-isopropylidene-ascorbic acid (1.1 g).

Example 1.5 - 3-O-(carboxypropyl)-5,6-isopropylidene

10 ascorbic acid

4.6 ml of 2N-NaOH was added to a solution of 3-O-(ethoxycarbonylpropyl)-5,6-isopropylidene-ascorbic acid (1.02 g) in 15 ml EtOH. After one hour, most of the ethanol was removed at reduced pressure and the residue
15 was diluted with water (10 ml), and acidified with dil-HCL (pH 3). The solution was then saturated with NaCl and extracted several times with ethyl acetate; the pooled extracts were then dried using MgSO_4 . Solvent was evaporated at reduced pressure to yield a viscous residue
20 containing 3-O-(carboxypropyl)-5,6-isopropylidene ascorbic acid (0.84 g). TLC: (Silica gel: $\text{CHCl}_3/\text{MeOH}/\text{HOAc}$ [5:1:0.1], $R_f=0.55$).

Example 1.6 - D-Nal-c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH₂

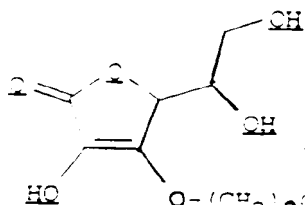
25 A solution of di-tertbutyl dicarbonate (0.36 g) in 10 ml DMF was added dropwise to a solution of D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH₂ acetate (2 g, BIM-23014) in 45 ml DMF. After two hours at room temperature, solvent was removed under reduced pressure
30 to yield a residue which was then chromatographed on silica gel (150 g) using $\text{CHCl}_3/\text{MeOH}$ (9:1) as an eluant. Appropriate fractions were pooled and solvents removed under reduced pressure to yield a residue containing D-

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Nal-c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH₂ (1.45 g).

TLC (silica gel: CHCl₃/MeOH [3:1], R_f=0.52).

Example 1.7 -



O-((CH₂)₃O)-D-Nal-c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH₂

- 0.2 ml diisopropylethylamine was added to a
- 5 solution of D-Nal-Cyclo-[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH₂ (300 mg), 3-O-(carboxypropyl)-5,6-isopropylidene ascorbic acid (56 mg) and HBTU (113 mg) in 5 ml DMF. The mixture was then stirred at room temperature overnight, and solvent was removed under reduced pressure. The
- 10 residue was partitioned between a mixture of ethyl acetate/MeOH and a saturated aqueous NaCl solution, and the ethyl acetate layer was washed with saturated aqueous NaCl, then saturated aqueous NaHCO₃, and then dried (MgSO₄). Solvent was evaporated under reduced pressure,
- 15 and the residue was subjected to preparative TLC using a CHCl₃/MeOH (8:1) mixture as a developing solvent. The appropriate UV-positive zone was isolated and extracted with CHCl₃/MeOH. Solvents were removed at reduced pressure to yield the above-identified product (0.20 g).
- 20 TLC (silica gel: CHCl₃/MeOH[5:1], R_f=0.54).

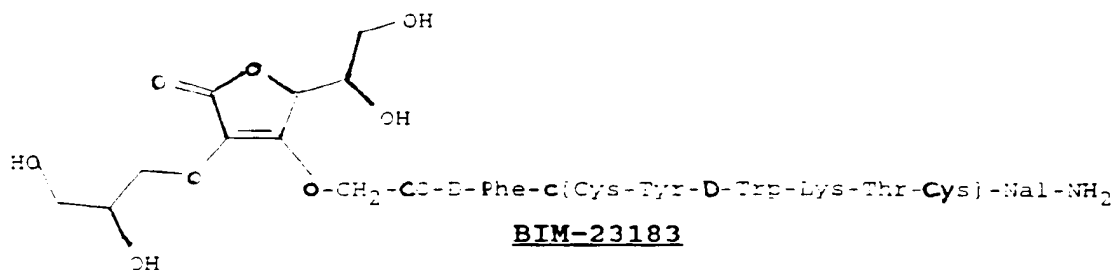
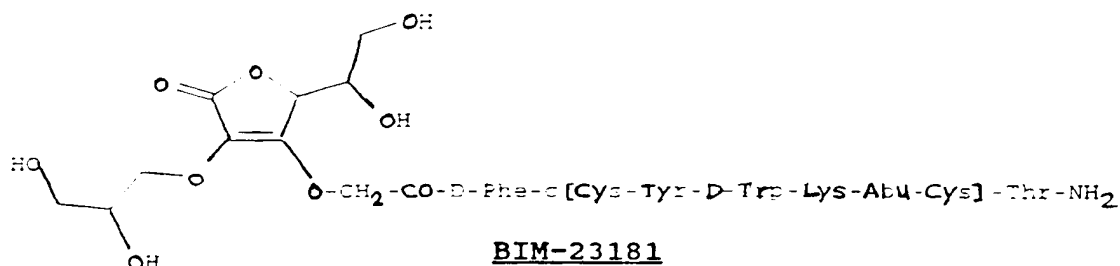
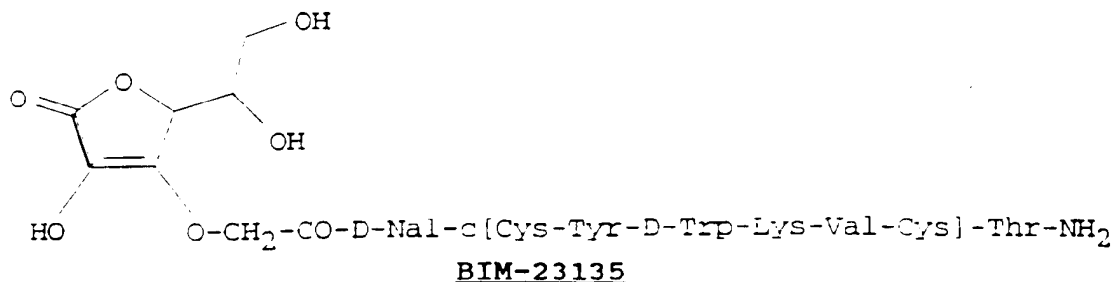
Example 1.8 - Removal of BOC Group

- The ascorbic acid derivative containing D-Nal-c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH₂ (95 mg) shown above was treated with 25% TFA in CHCl₃ for 45 min. at
- 25 room temperature. Volatile substances were removed under reduced pressure to yield a dried residue which was purified using Vydac C₁₈ HPLC and CH₃CN/0.1% aqueous TFA. The final yield was 90 mg (FAB-MS (m/e) 1341).

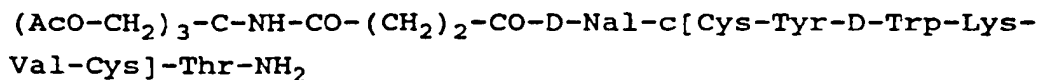
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Example 1.9 - Other Embodiments

The following somatostatin derivatives were also synthesized in an analogous manner:

Example 2 - Synthesis of BIM-23107

The following somatostatin derivative, also referred to as BIM-23107, was synthesized in accordance to the invention.

Example 2.1 - $(\text{AcO}-\text{CH}_2)_3-\text{C}-\text{NH}-\text{CO}-(\text{CH}_2)_2-\text{CO}-\text{D-Nal}-\text{c}[\text{Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys}]-\text{Thr}-\text{NH}_2$

0.03 ml DIEA was added to an ice-cooled solution of 2-N-(succinyl)amino-2-(acetoxymethyl)-1,3-propanediol

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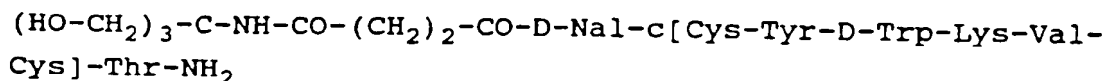
diacetate (83 mg) and HBTU (92 mg) in 2 ml of DMF. After stirring at 0-5° C for 30 minutes, a solution of D-Nal-c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH₂ (100 mg) in 2 ml DMF, containing 0.03 ml DIEA, was added. The mixture
5 was first stirred at 0-5° C for one hour and then stirred at room temperature overnight. The solvent was removed at reduced pressure to yield a dried residue which was partitioned between ethyl acetate and aqueous saturated NaCl, and the EtOAc layer washed with aqueous 5% NaHCO₃,
10 and finally aqueous saturated NaCl; the resulting solution was then dried using MgSO₄. The solvent was evaporated under reduced pressure leaving a residue containing (AcO-CH₂)₃-C-NH-CO-(CH₂)₂-CO-D-Nal-c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH₂ (0.14 gm). TLC (Silica Gel
15 : CHCl₃/MeOH/HOAc = 4:1:0.1, R_f=0.82).

Example 2.2 - Removal of BOC group

30 mg of the above-identified compound was treated with 50% TFA in CHCl₃ for 45 minutes at room temperature; volatile substances were then removed at reduced pressure
20 to yield a residue. Traces of TFA were co-evaporated with ethanol several times and the residue was titrated with ether and then dried to yield 30 mg of the product (30 mg). TLC (Silica gel: CHCl₃/MeOH/HOAc = 3:1:1, R_f=0.24).

25 Example 2.3 - Other Embodiments

The following somatostatin derivatives were also synthesized in an analogous manner.



30

BIM-23158

- 25 -

$(\text{HO}-\text{CH}_2)_3-\text{C}-\text{NH}-\text{CO}-(\text{CH}_2)_2-\text{CO}-\text{D-Phe}-\text{c}[\text{Cys-Tyr-D-Trp-Lys-Thr-Cys}]-\text{Nal}-\text{NH}_2$

BIM-23167

$(\text{HO}-\text{CH}_2)_3-\text{C}-\text{NH}-\text{CO}-(\text{CH}_2)_2-\text{CO}-\text{D-Phe}-\text{c}[\text{Cys-Tyr-D-Trp-Lys-Abu-Cys}]-\text{Thr}-\text{NH}_2$

BIM-23173

$(\text{HO}-\text{CH}_2)_3-\text{C}-\text{NH}-\text{CH}_2-\text{CO}-\text{D-Phe}-\text{c}[\text{Cys-Tyr-D-Trp-Lys-Thr-Cys}]-\text{Nal}-\text{NH}_2$

BIM-23179

10 $(\text{HO}-\text{CH}_2)_3-\text{C}-\text{NH}-\text{CH}_2-\text{CO}-\text{D-Phe}-\text{c}[\text{Cys-Tyr-D-Trp-Lys-Abu-Cys}]-\text{Thr}-\text{NH}_2$

BIM-23182Example 3 - Synthesis of BIM-23201

The following somatostatin derivative, also
15 referred to as (BIM-23201), was synthesized in accordance with the present invention.

$(\text{HO}-\text{CH}_2)_3-\text{C}-\text{CH}_2-\text{D-Phe}-\text{c}[\text{Cys-Tyr-D-Trp-Lys-Thr-Cys}]-\text{Nal}-\text{NH}_2$

Example 3.1 - $(\text{HO}-\text{CH}_2)_3-\text{C}-\text{CH}_2-\text{D-Phe}-\text{c}[\text{Cys-Tyr-D-Trp-Lys-Thr-Cys}]-\text{Nal}-\text{NH}_2$

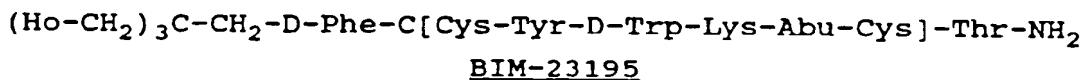
20 Two grams of 3Å molecular sieve followed by NaCNBH₃ (36 mg) were added portion-wise in 15 minute increments to a solution of D-Phe-c[Cys Tyr (OBt)-D-Trp-Lys(BOC)-Thr(OBT) Cys] Nal-NH₂ (250 mg) and tris (acetoxymethyl)acetaldehyde (120 mg) obtained by
25 oxidation of triacetyl penta-erythritol with pyridinium dichromate or DMSO/oxalyl chloride/triethylamine) in methanol (10 ml) containing 10% acetic acid. The mixture

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was then stirred at room temperature for 30 minutes and heated for 4 hours. After filtration, the residue was partitioned between ethyl acetate and water. The ethyl acetate layer was washed with water, then aqueous NaHCO_3 ,
 5 and then dried (MgSO_4). The solvent was evaporated under reduced pressure to yield a residue (0.4 g) which was then dissolved in methanol (5 ml), treated with a NaOMe/MeOH solution (pH 10), stirred for 1 hour and finally neutralized with 1 N HCl to pH 5-6. After
 10 evaporation of solvent, the residue was dissolved in 90% aqueous TFA (5 ml) and stirred for 30 minutes. Volatiles were removed at reduced pressure and traces of TFA and water in the resulting residue were removed by co-evaporation with alcohol (2x). The residue was dried,
 15 then titrated with ether, and finally purified by HPLC using conditions similar to those described earlier, to yield 41 mg of $(\text{HO-CH}_2)_3\text{-C-CH}_2\text{-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH}_2$ as a colorless solid. MS (m/e) 1262.8.

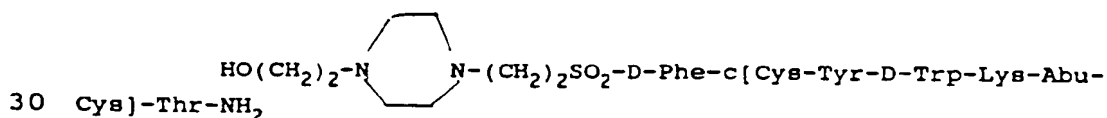
Example 3.2 - Other Embodiments

20 The following somatostatin derivative, also referred to as BIM-23195, was synthesized in an analogous manner.



25 Example 4 - Synthesis of BIM-23197

The following somatostatin derivative, also referred to as BIM-23197, was synthesized in accordance with the invention.



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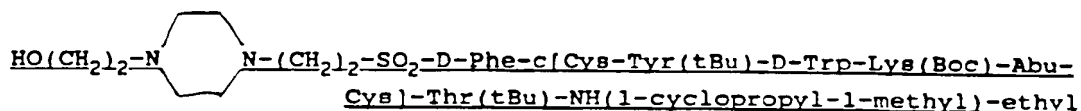
Example 4.1 - 2-Bromoethanesulfonyl Chloride

Na 2-Bromoethanesulfonate (4.0 g) was treated with PCl_5 (11.8 g) while cooling in an ice bath. After reaching the liquid phase, the solution was heated at 90-
5 120 °C for 1.5 hours in oil, cooled to room temperature, poured into 50 g of crushed ice, and then stirred for 15 min. The mixture was extracted with CH_2Cl_2 (3 x 30 ml) and combined extracts were washed with H_2O (2 x), 5% NaHCO_3 (2 x), and H_2O (2 x) again. Drying over anhydrous
10 MgSO_4 and distillation under reduced pressure gave 2-bromoethanesulfonyl chloride as a colorless liquid (1.95 g, 42-44 °C/1 mm Hg).

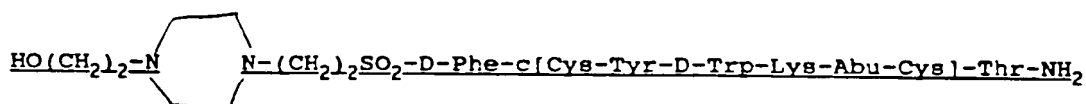
Example 4.2 - Br-(CH₂)₂-SO₂-D-Phe-c[Cys-Tyr(tBu)-D-Trp-Lys(Boc)-Abu-Cys]-Thr(tBu)-NH(1-cyclopropyl-1-methyl)-ethyl
15 ethyl

A solution of 2-bromoethane sulfonyl chloride (30 mg) in DMF (1 ml) was added dropwise to a solution of H-D-Phe-c[Cys-Tyr(tBu)-D-Trp-Lys(Boc)-Abu-Cys]-Thr(tBu)-(1-cyclopropyl-1-methyl)-ethyl (150 mg) and DIEA (55 mg) in
20 DMF (2 ml) under N_2 at 0°C. The reaction mixture was stirred at 0-5 °C for 3 hours; solvent was then removed under reduced pressure. The residue was dissolved in ethyl acetate and washed with 5% citric acid (2 x), 5% NaHCO_3 (2 x) and brine (2 x). The solution was then
25 dried over anhydrous MgSO_4 , filtered, and condensed to dryness under reduced pressure. The product was further purified by a short silica gel column eluted with ethyl acetate. Fractions containing the product were pooled and the solvent was removed under reduced pressure,
30 giving 105 mg of Br-(CH₂)₂-SO₂-D-Phe-c[Cys-Tyr(tBu)-D-Trp-Lys(Boc)-Abu-Cys]-Thr(tBu)-NH(1-cyclopropyl-1-methyl)-ethyl as a slightly yellow solid. (Silica gel, $\text{CHCl}_3/\text{MeOH}/\text{HOAc}$ (9:1:0.1), $R_f=0.36$).

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Example 4.3 -

A solution of Br-(CH₂)₂-SO₂-D-Phe-c[Cys-Tyr(tBu)-
 5 D-Trp-Lys(Boc)-Abu-Cys]-Thr(tBu)-NH(1-cyclopropyl-1-
 methyl)-ethyl (100 mg) and 2-hydroxyethylpiperazine (55
 mg) in 2 ml of 1-propanol was refluxed under N₂ for 2.5
 hours. The solution was then cooled to room temperature,
 and the solvent was removed under reduced pressure. The
 10 residue was then dissolved in ethyl acetate containing 5%
 MeOH and washed with brine (3 x). Finally, the solution
 was dried over anhydrous MgSO₄, filtered and condensed to
 dryness under reduced pressure, resulting in 110 mg of
 the above-identified solid. Without further
 15 purification, this compound was used directly in the next
 step.

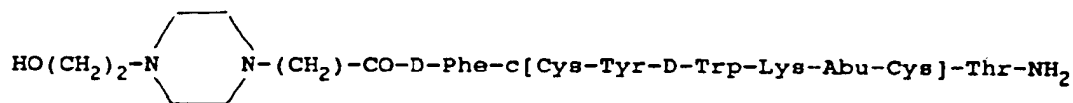
Example 4.4 -

110 mg of the protected somatostatin derivative
 20 obtained in the previous step was dissolved in 10 ml of
 90% TFA aqueous solution, and stirred at room temperature
 under N₂ for one hour. TFA and H₂O were removed under
 reduced pressure, and the residue was titrated with cold
 ether (3 x 10 ml). A slightly yellow solid was obtained;
 25 this material was further purified on preparative reverse
 phase HPLC, eluting with: 1) a NH₄OAc aqueous solution;
 and, 2) an HOAc aqueous solution. Lyophilization of the
 pooled fractions containing the above-identified product
 gave a white solid. (18 mg. ESI-MS, ((m+1)/e) 1252.7).

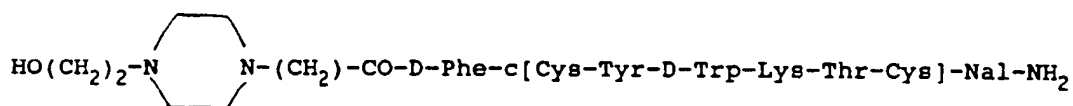
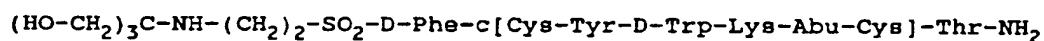
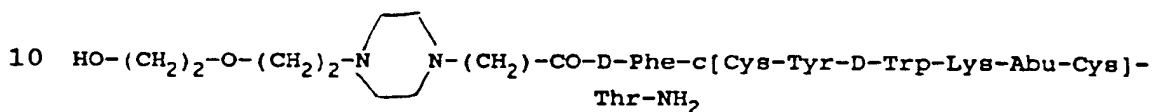
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Example 4.5 - Other Embodiments

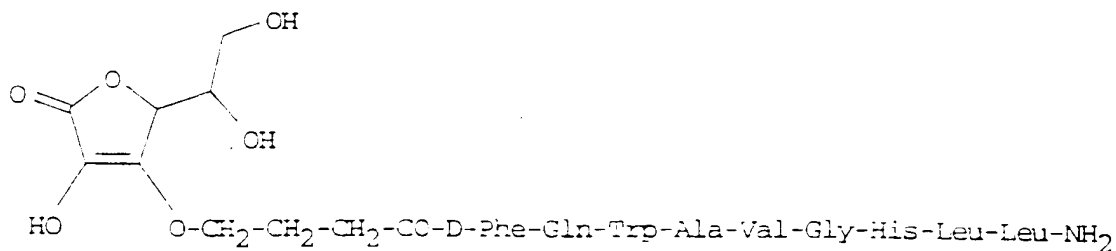
The following somatostatin derivatives were also synthesized in an analogous manner:



5

BIM-23190BIM-23191BIM-23196BIM-23202Example 5 - Synthesis of Bombesin Derivatives

15 The following bombesin derivative, also referred to as BIM-26333, was synthesized in an analogous manner as described above:



- 30 -

Other peptide derivatives of the invention can be synthesized in an analogous manner, using synthetic modifications known in the art.

Results of Assays of Test Peptides

5 Example 6 - Binding Assays

In order to demonstrate the binding affinity of somatostatin (SRIF) analogs to the somatostatin receptor, the purified compounds described above were tested in somatostatin binding assays involving measurements of the
10 in vitro inhibition of the binding of [^{125}I -Tyr 11]SRIF-14 to rat AR42J pancreas membranes. As indicated in Table I, purified somatostatin analogs of this invention demonstrated high binding affinities to these receptors. Additionally, the molecular weight, determined by mass
15 spectrometry and estimated from the molecular structure, is listed in the table for each somatostatin derivative.

Similarly, the purified bombesin analog described above was tested in a bombesin binding assay. The binding assay consisted of measurements of the in vitro
20 inhibition of the binding of [^{125}I -Tyr 11] bombesin to rat AR42J pancreas membranes; from the assay, the binding affinity of the bombesin analog to the GRP receptor was determined to be about 21 nM.

25 Example 7 - Growth Hormone (GH) Inhibition Assay

Groups of five male Sprague Dawley rats (each having a weight between 250-300 g) were injected s.c. with a somatostatin derivative or saline. Thirty minutes prior to the selected post-drug time periods shown in
30 table II (2 hours, 4 hours, 6 hours, 8 hours), rats were anesthetized with Nembutal i.p. (50 mg/kg). Fifteen minutes following anesthesia, an aliquot of blood was withdrawn by cardiac puncture over heparin to measure basal GH. Additionally, a s.c. injection of D-Ala 2 -GRF
35 (10 $\mu\text{g/kg}$) was given. Fifteen minutes later, blood was

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withdrawn to quantitate the stimulated GH, which was measured in plasma using a radioimmunoassay supplied by NIADDKD. The percentage of GH inhibition was calculated from differences obtained between basal and stimulated GH values.

Table II shows the effect of various purified somatostatin analogs as a function of time. The efficacy of D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH₂ (BIM-23060) in inhibiting growth hormone in rats is compared with other somatostatin derivatives (BIM-23167, BIM-23179, and BIM-23181) of the invention. All derivatives demonstrate a surprising prolonged duration of action which decreases in a time-dependent fashion.

Additional experiments were conducted on D-Phe-c[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH₂, a somatostatin analog, and BIM-23190, BIM-23195 and BIM-23197, to determine the ED₅₀ (i.e., the concentration of each compound required to inhibit fifty percent of growth hormone release after a specified time) of the respective compound. Experiments were conducted at a dose range of between 25 µg/kg and 0.25 µg/kg. Table III shows the surprising improvement of the somatostatin derivatives over the unmodified peptide at the various time intervals, indicating the time-dependent inhibition of stimulated GH release by the compounds of the invention.

Example 8 - Antiproliferative Assay

The purified somatostatin analogs described above were also tested for activity against rapidly proliferating cells. Table IV describes the effect of these peptides on the growth of AR42J rat pancreas tumor cells. Unlike natural somatostatin, the derivatives of the invention demonstrate substantial anti-proliferative activity. Referring now to Fig. 1, both BIM-23014C (a somatostatin analog) and BIM-23118 (a derivative of BIM-

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23014) inhibit the growth of AR42J rat pancreas tumor cells in a concentration-dependent fashion, with BIM-23118 being the more effective of the two compounds. Both compounds inhibit tumor cell growth to a greater
5 extent than unmodified somatostatin analogs at equivalent concentrations.

Example 9 - Thymidine Uptake Assay

In this assay, stock cultures of Swiss 3T3 cells are grown in Dulbecco's Modified Eagles Medium (DMEM) and
10 supplemented with 10% fetal calf serum in a humidified atmosphere of 10% CO₂ and 90% air at 37°C. Cells were then seeded into 24-well cluster trays and used four days after the last change of medium. In order to arrest cells in the G1/G0 phase of the cell cycle, the a serum-
15 free DMEM was used 24 hours prior to the thymidine uptake assay; cells were then washed twice with 1 ml aliquots of DMEM (-serum, 0.5 µM) and [methyl-³H] thymidine (20Ci/mmoles, New England Nuclear). Bombesin derivatives were initially tested at 0.001, 0.01, 0.1, 1, 10, 100,
20 100 nM. After 28 hours at 37°C, [methyl-³H] thymidine incorporation into acid-insoluble pools was assayed as follows. Cells were first washed twice with ice-cold 0.9% NaCl (1 ml aliquots); acid-soluble radioactivity was then removed by 30-minute incubation at 40°C with 5%
25 trichloroacetic acid (TCA). The cultures were then washed once (1 ml) with 95% ethanol and solubilized by a 30-minute incubation with 1 ml of 0.1N NaOH. The solubilized material was transferred to vials containing 10 ml ScintA (Packard), and the radioactivity determined
30 by liquid scintillation spectrometry. This assay demonstrates the ability of the bombesin derivatives to stimulate thymidine uptake into the cells. The EC₅₀ was

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calculated to be 0.48 nm, thus demonstrating that the bombesin derivatives of the invention are potent simulators of thymidine uptake.

Methods of Use

- 5 The peptide derivatives of the invention may be administered to a mammal, particularly a human, in one of the traditional modes (e.g., orally, parenterally, transdermally, or transmucosally), in a sustained-release formulation using a biodegradable, biocompatible polymer,
- 10 or by on-site delivery (e.g., in the case of an anti-cancer bombesin or somatostatin derivatives, to the lungs) using micelles, gels and liposomes. Dosages are generally the same as those currently used for therapeutic peptides in humans.
- 15 Additionally, the peptide derivatives of the invention are suitable for the improved treatment of diseases which are susceptible to treatment by the corresponding unmodified peptide. In particular, the somatostatin derivatives described above are suitable for
- 20 the treatment of cancer, acromegaly, pancreatitis, trauma induced proliferation, diabetes, diabetic retinopathy, restenosis following angioplasty, AIDS, neurogenic inflammation, arteritis, and gastrointestinal problems including diarrhea.

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**TABLE I-IN VITRO BINDING AFFINITIES AND MOLECULAR WEIGHTS
OF SOMATOSTATIN PEPTIDE DERIVATIVES**

		MW TEST	MW CALC	IC ₅₀ nM
	SRIF - 14	-	-	0.17
	SRIF - 28	-	-	0.23
5	BIM - 23107	1340.4	1340.40	0.30
	BIM - 23118	1313.5	1313.52	0.30
	BIM - 23135	1426.2	1426.64	2.52
	BIM - 23158	1299.6	1299.54	0.33
	BIM - 23167	1347.6	1347.55	0.09
10	BIM - 23173	1235.5	1235.46	0.11
	BIM - 23179	1305.9	1305.55	0.12
	BIM - 23181	1435.0	1434.62	0.25
	BIM - 23182	1193.8	1193.42	0.12
	BIM - 23183	1323.0	1322.49	0.22
15	BIM - 23190	1202.8	1202.47	0.20
	BIM - 23191	1314.9	1314.61	0.08
	BIM - 23195	1150.8	1150.39	0.08
	BIM - 23196	1243.7	1243.50	0.09
	BIM - 23197	1252.7	1252.55	0.29
20	BIM - 23201	1262.8	1262.53	0.14
	BIM - 23202	1247.0	1246.53	0.18

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**TABLE II-INHIBITION OF STIMULATED GROWTH HORMONE RELEASE
IN RATS BY SOMATOSTATIN PEPTIDE DERIVATIVES**

INHIBITION (PERCENTILE CONTROL) 25 µG/KG

	2 Hours	4 Hours	6 Hours	8 Hours
BIM-23060	86.39	64.96	47.62	38.15
BIM-23167	92.67	79.54	59.72	50.14
BIM-23179	92.79	63.85	67.78	68.26
BIM-23181	99.24	77.07	60.56	56.12

**TABLE III-INHIBITION OF STIMULATED GROWTH HORMONE RELEASE
IN RATS BY SOMATOSTATIN PEPTIDE DERIVATIVES ADMINISTERED**

10 S.C.

ED 50 (µg/kg)

	2 Hours	4 Hours	6 Hours	8 Hours
BIM-23023	0.48	1.11	2.26	4.32
BIM-23190	0.68	0.57	0.76	1.04
BIM-23195	1.19	3.13	2.08	3.23
BIM-23197	1.01	0.59	1.14	1.59

15

SUBSTITUTE SHEET (RULE 26)

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TABLE IV-ANTIPROLIFERATIVE ACTIVITY OF
SOMATOSTATIN PEPTIDE DERIVATIVES

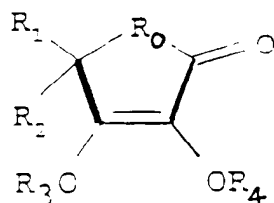
CELL GROWTH (PERCENT OF CONTROL) ¹		
5	SRIF - 14	91.3
	SRIF - 28	98.0
	BIM-23014C	74.1
	BIM-23107	67.5
	BIM-23109	72.1
10	BIM-23118	61.0
	BIM-23135	62.9
	BIM-23167	60.2
	BIM-23173	67.9
	BIM-23181	69.1
15	BIM-23182	68.7
	BIM-23183	69.1
	BIM-23195	69.2
	BIM-23197	66.4

¹ Concentration 100 nM, AR42J Rat Pancreas Tumor Cells
after 8 days.

20 What is claimed is:

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1. A peptide derivative comprising:
 a biologically active peptide moiety, and
 at least one substituent attached to said peptide
 moiety, wherein said substituent is selected from the
 group consisting of Compounds I, II and III, wherein
 5 Compound I is:

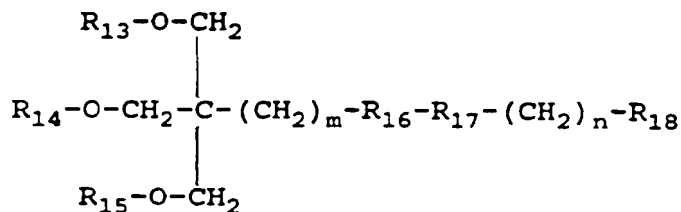


wherein :

- R_0 is O, S, or NR_5 , wherein R_5 is H or (C_1-C_6) alkyl;
- 10 each R_1 and R_2 , independently, is H, $(CH_2)_mOR_6$, or $CH(OR_7)CH_2OR_8$, wherein R_6 is H or (C_2-C_7) acyl, and each R_7 and R_8 , independently, is H, (C_2-C_7) acyl, or $C(R_9)(R_{10})$, wherein each R_9 and R_{10} , independently, is H or (C_1-C_6) alkyl;
- 15 or each R_1 and R_2 is $=CHCH_2OR_{11}$, wherein in R_1 and R_2 independently, R_{11} is H or (C_2-C_7) acyl, and m is an integer between 1 and 5, inclusive; and
- one of R_3 or R_4 is $(CH_2)_nR_{12}$ or $(CH_2)_nCH(OH)R_{12}$,
 20 wherein R_{12} is CO, CH_2 , or SO_2 , and n is an integer between 1 and 5, inclusive;
- and the remaining R_3 or R_4 is H, (C_1-C_6) hydroxyalkyl, or (C_2-C_7) acyl;
 and

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Compound II is:



5 wherein:

each R_{13} , R_{14} and R_{15} , independently, is H or (C_2 - C_{24}) acyl;

R_{16} is NH or absent;

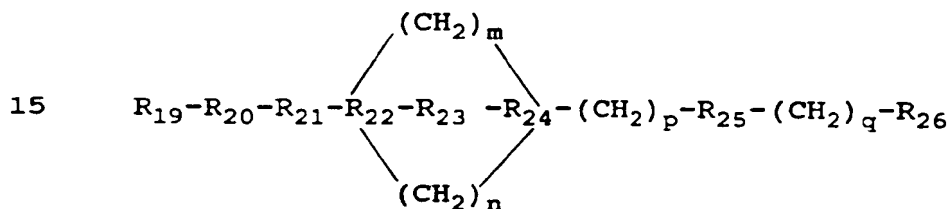
R_{17} is CO, O, or absent;

10 R_{18} is CO, CH_2 , SO_2 , or absent;

m is an integer between 1 and 5, inclusive;

n is an integer between 0 and 5, inclusive; and

Compound III is:



wherein:

R_{19} is H, NH_2 , an aromatic functional group, OH, (C_1 - C_6)hydroxyalkyl, $H(R_{27})(R_{28})$,

20 SO_3H , or absent; wherein each R_{27} and R_{28} , independently, is H or (C_1 - C_6) alkyl;

R_{20} is O or absent;

R_{21} is (C_1 - C_6)alkyl or absent;

R_{22} is N, O, C, or CH;

25 $-R_{23}-$ is (C_1 - C_6)alkyl or absent;

R_{24} is N, CH, or C;

R_{25} is NH, O, or absent;

R_{26} is SO_2 , CO, CH_2 , or absent;

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m is an integer between 0 and 5, inclusive;
n is an integer between 0 and 5, inclusive;
p is an integer between 0 and 5, inclusive; and
q is an integer between 0 and 5 inclusive;

5 wherein said peptide moiety is attached to each of
said substituents by a CO-N, CH₂-N, or SO₂-N bond between
said substituent and a nitrogen atom of the N-terminus or
a side chain of said peptide moiety.

2. The peptide derivative of claim 1, wherein
10 said substituent is Compound I.

3. The peptide derivative of claim 2, wherein
R₁₂ is CH₂ or SO₂.

4. The peptide derivative of claim 1, wherein
said substituent is Compound II.

15 5. The peptide derivative of claim 4, wherein
R₁₈ is CH₂ or SO₂.

6. The peptide derivative of claim 5, wherein
R₁₃, R₁₄, and R₁₅ are H, and R₁₇ is absent.

7. The peptide derivative of claim 6, wherein
20 said substituent is (HOCH₂)₃C-NH-(CH)₂-SO₂ or (HOCH₂)₃C-
CH₂.

8. The peptide derivative of claim 1, wherein
said substituent is Compound III.

9. The peptide derivative of claim 8, wherein
25 -R₂₃- is (C₁-C₆)alkyl; R₂₂ is N, C, or CH; and R₂₄ is C.

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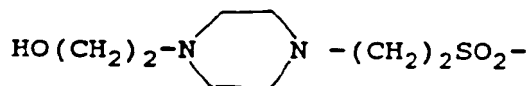
10. The peptide derivative of claim 8, wherein R_{22} is O; R_{19} , R_{20} , R_{21} , and $-R_{23}-$ are absent; and the sum of m and n is 3, 4, or 5.

11. The peptide derivative of claim 8, wherein $-R_{23}-$ is absent.

12. The peptide derivative of claim 11, wherein at least one of R_{22} and R_{24} is N.

13. The peptide derivative of claim 12, wherein both R_{22} and R_{24} are N.

10 14. The peptide derivative of claim 13, wherein said substituent is one of:



and



15 15. The peptide derivative of claim 1, wherein said peptide moiety is selected from the group consisting of: somatostatin, bombesin, calcitonin, calcitonin gene related peptide (CGRP), amylin, parathyroid hormone (PTH), gastrin releasing peptide (GRP), melanocyte
20 stimulating hormone (MSH), adrenocorticotrophic hormone (ACTH), parathyroid related peptide (PTHrP), luteinizing hormone-releasing hormone (LHRH), growth hormone releasing factor (GHRF), growth hormone releasing peptide (GHRP), cholecystokinin (CCK), glucagon, Bradykinin,
25 glucagon-like peptide (GLP), gastrin, enkephalin, neuromedins, endothelin, substance P, neuropeptide Y (NPY), peptide YY (PYY), vasoactive intestinal peptide (VIP), guanylin, pituitary adenylate cyclase activating

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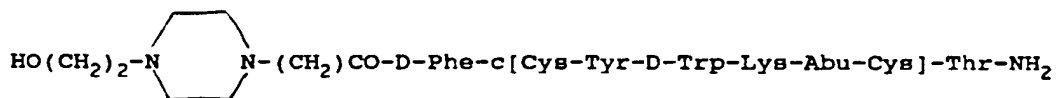
polypeptide (PACAP), beta-cell tropin, adrenomedulin, and derivatives, fragments, and analogs thereof.

16. The peptide derivative of claim 15, wherein said peptide moiety is somatostatin or a derivative, fragment, or analog thereof.

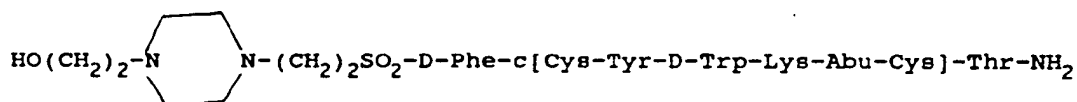
17. The peptide derivative of claim 16, wherein said somatostatin analog is one of: H-D-Phe-c[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH₂, H-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH₂, and H-D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH₂.

18. The peptide derivative of claim 15, wherein said peptide moiety is bombesin or a derivative, fragment or analog thereof.

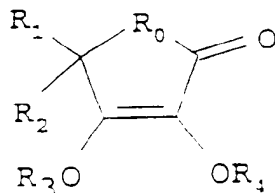
19. The peptide derivative of claim 1, wherein said peptide derivative is one of:



and



20. A dimeric peptide derivative, comprising:
two biologically active peptide moieties, and
at least one substituent attached to one of said peptide moieties, wherein said substituent is one of Compounds IV and V, wherein Compound IV is:

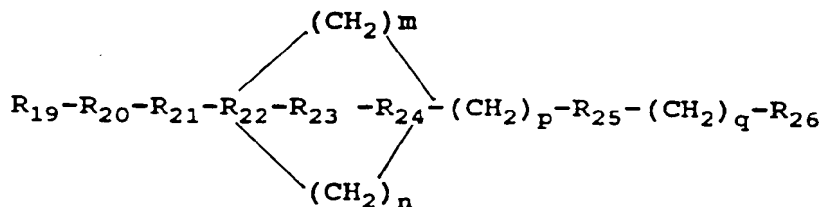


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wherein :

- R_0 is O, S, or NR_5 , wherein R_5 is H or (C_1-C_6) alkyl;
- each R_1 and R_2 , independently, is H, $(CH_2)_mOR_6$, or $CH(OR_7)CH_2OR_8$, wherein R_6 is H or (C_2-C_7) acyl, and each R_7 and R_8 , independently, is H, (C_2-C_7) acyl, or $C(R_9)(R_{10})$, wherein each R_9 and R_{10} , independently, is H or (C_1-C_6) alkyl;
- or each R_1 and R_2 is $=CHCH_2OR_{11}$, wherein R_{11} in R_1 and R_2 , independently, is H or (C_2-C_7) acyl, and m is an integer between 1 and 5, inclusive; and each R_3 or R_4 , independently, is $(CH_2)_nR_{12}$ or $(CH_2)_nCH(OH)R_{12}$, wherein R_{12} is CO, CH_2 , or SO_2 , and n is an integer between 1 and 5, inclusive; and,

- Compound V is:



wherein:

- R_{19} is SO_2 , CO, or CH_2 ;
- R_{20} is O or absent;
- R_{21} is (C_1-C_6) alkyl or absent;
- R_{22} is N, CH, O, or C;
- $-R_{23}-$ is (C_1-C_6) alkyl or absent;
- R_{24} is N, CH, or C;
- R_{25} is NH, O, or absent;
- R_{26} is SO_2 , CO, CH_2 , or absent;
- m is an integer between 0 and 5, inclusive;
- n is an integer between 0 and 5, inclusive;

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p is an integer between 0 and 5, inclusive;
q is an integer between 0 and 5, inclusive; and
wherein at least one of said peptide moieties is
attached to each of said substituents by a CO-N, CH₂-N,
5 or SO₂-N bond between said substituent and a nitrogen
atom of either the N-terminus or a side chain of one of
said peptide moieties.

21. The dimeric peptide derivative of claim 20,
wherein -R₂₃- is (C₁-C₆)alkyl; R₂₂ is N, C or CH; and R₂₄
10 is C.

22. The dimeric peptide derivative of claim 20,
wherein R₂₂ is O; R₁₉, R₂₀, R₂₁ and -R₂₃- are absent; and
the sum of m and n is 3, 4, or 5.

23. A method of treating a disease of a patient,
15 comprising the step of administering to said patient a
therapeutic amount of the peptide derivative of claim 1.

24. The method of claim 23, wherein said peptide
moiety is somatostatin or an analog thereof.

25. The method of claim 23, wherein said disease
20 is cancer.

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Effect of Somatostatin Analogs on the Proliferation of AR42J Rat Pancreatic Tumor Cells

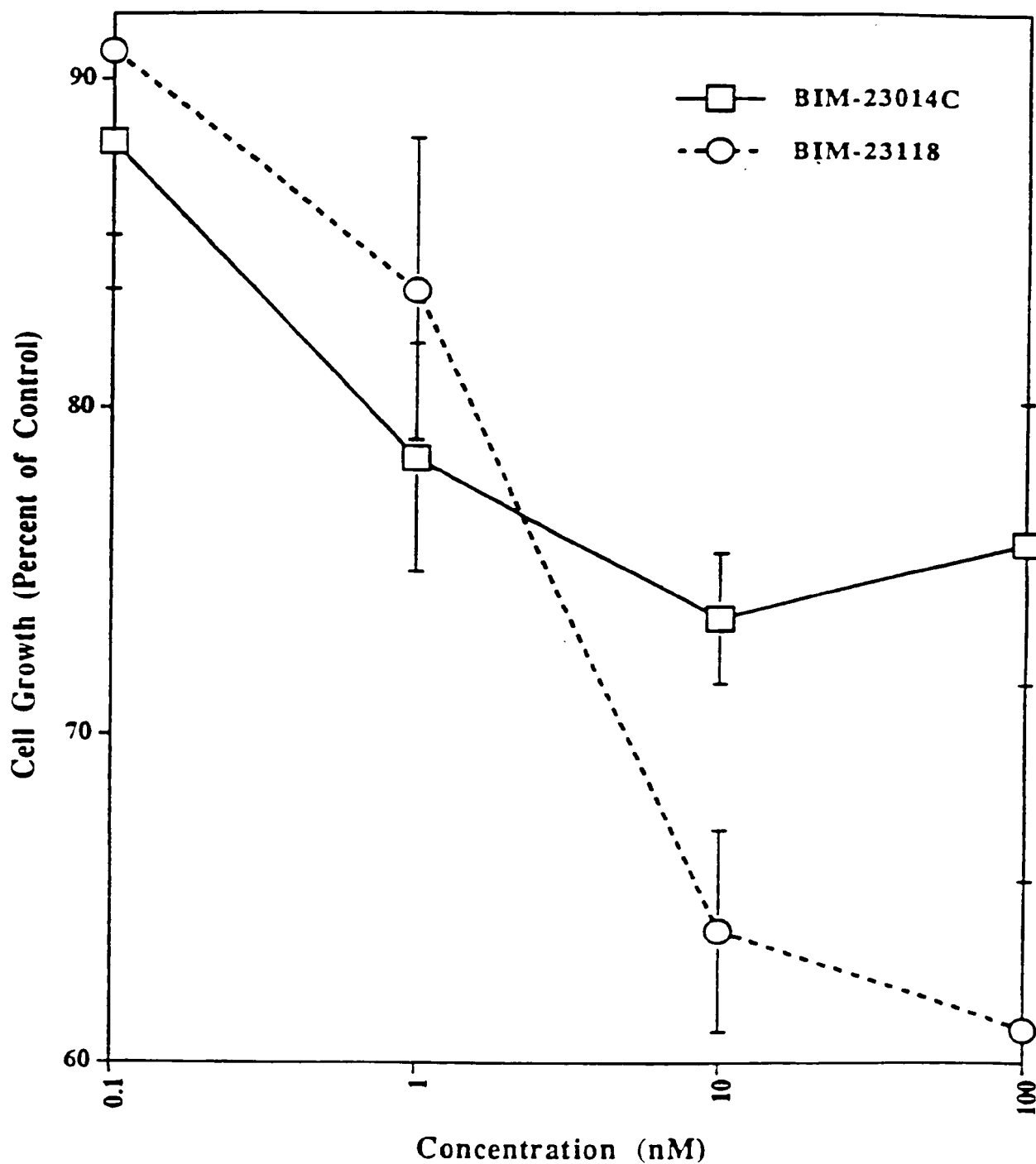


Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/08875

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 13, 14, 15, 16, 21; 530/302, 306, 307, 308, 311, 324, 345, 350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A, 88/02756 (SANDOZ AG) 21 April 1988, see entire document.	1-25
X	WO, A, 89/09786 (ALBERT ET AL) 19 October 1989, see entire document.	1-25
X	US, A, 4,837,303 (JUNG) 06 June 1989, see entire document.	1-25

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"G" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

22 SEPTEMBER 1994

Date of mailing of the international search report

17 OCT 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

CAROL A. SALATA

Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08875

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

C07K 5/04, 7/10, 7/34, 7/36, 7/44, 7/26, 7/38, 7/12; A61K 37/24, 37/28, 37/40

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/12, 13, 14, 15, 16, 21; 530/302, 306, 307, 308, 311, 324, 345, 350

1/1

Effect of Somatostatin Analogs on the Proliferation of AR42J Rat Pancreatic Tumor Cells

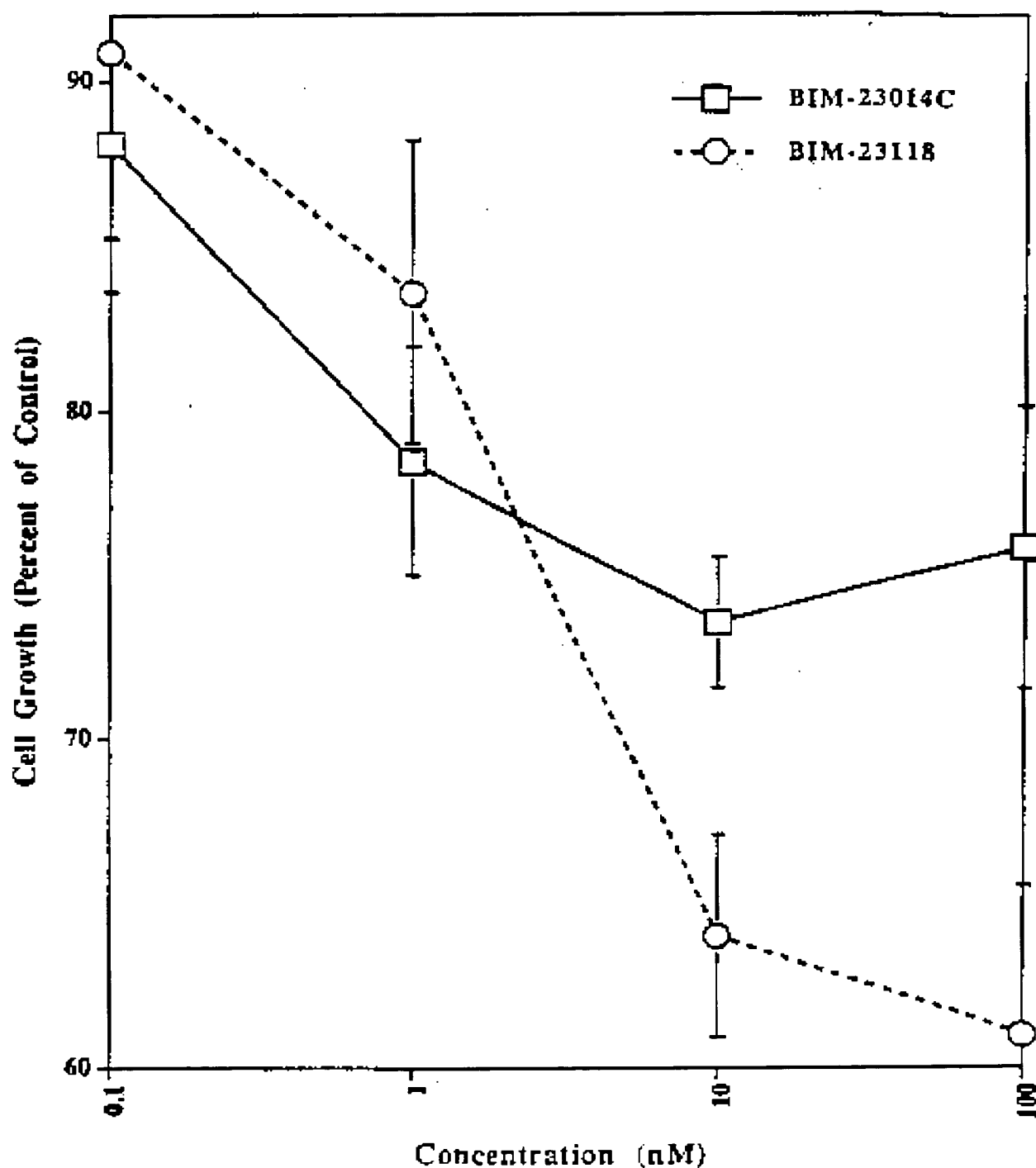


Fig. 1

